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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED OFFICE (DO/US)

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NEW COMPOUNDS

Title of Invention

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Applicant(s) for DO/US

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To the United States Designated Office (DO/US):

- I. Accompanying this transmittal letter are certain items which are required under 35 U.S.C. 371 in order that United States National processing of the above identified International application may commence:
- (X) at the expiration of the applicable time limit under PCT Articles 22 and 39(1) according to the provisions of 35 U.S.C. 371(b).
  - ( ) as soon as possible upon receipt of this express request under 35 U.S.C. 371(f).

b. (X) is submitted herewith as follows:

c. ( ) has been transmitted

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- i. ☐ by the International Bureau. Date of mailing of the application (from form PCT/IB/308): \_\_\_\_\_ A copy of form PCT/IB/308 is enclosed.
- ii. ☐ by applicant on (date) \_\_\_\_\_.
3. A translation of the International application into the English language [35 U.S.C. 371(c)(2)]:
- a. ☐ is transmitted herewith.
- b. ☒ is not required as the application was filed in English.
- c. ☐ was previously transmitted by applicant on (date) \_\_\_\_\_.
4. Amendments to the claims of the International application under PCT Article 19 [35 U.S.C. 371(c)(3)]:
- a. ☐ are transmitted herewith.
- b. ☐ have been transmitted
- i. ☐ by the International Bureau. Date of mailing of the amendments (from form PCT/IB/308): \_\_\_\_\_.
- ii. ☐ by applicant on (date) \_\_\_\_\_.
- c. ☒ have not been transmitted as
- i. ☐ no notification has been received that the International Searching Authority has received the Search Copy.
- ii. ☐ the Search Copy was received by the International Searching Authority but the Search Report has not yet issued. Date of receipt of Search Copy (from form PCT/ISA/202): \_\_\_\_\_.
- iii. ☐ applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210): \_\_\_\_\_.

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- iv. ☒ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.
5. A Translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)]:
- a. ☐ is transmitted herewith.
- b. ☐ is not required as the amendments were made in the English language.
- c. ☒ has not been transmitted for reasons indicated at point I.4.b. or c. above.
6. An executed declaration for patent application of the inventor [35 U.S.C. 371(c)(4)] complying with 35 U.S.C. 115:
- a. ☐ was previously submitted by applicant on (date)  
\_\_\_\_\_
- b. ☒ is submitted herewith;  
and such oath or declaration
- i. ☒ is attached to the application.
- ii. ☒ identifies the application and any amendments under PCT Article 19 which were transmitted as stated in points 1.2.b. or c. and 1.4. and states that they were reviewed by the inventor as required by 37 CFR 1.70.
- c. ☐ will be submitted subsequently.

II. Concerning other documents:

1. An International Search Report or Declaration under PCT Article 17(2)(a):
- a. ☐ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): \_\_\_\_\_ A copy of form PCT/IB/308 is enclosed
- b. ☐ is not required as the application was searched by the United States International Searching Authority.
- c. ☐ A copy of the International Search Report is transmitted herewith.

- d. ( ) has been submitted by applicant on (date) \_\_\_\_\_.
2. A Statement of prior art under 37 CFR 1.97 and 1.98:
- a. ( ) is transmitted herewith including copies of the references cited on the attached form PTO-1449. Also enclosed is a copy of the International-Type Search Report (PCT/ISA/201/SE), issued in the Swedish priority application.
- b. ( ) will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
- c. ( ) was previously submitted by applicant on \_\_\_\_\_, in application serial no. \_\_\_\_\_.
3. (X) An Assignment is transmitted herewith for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
- a. (X) Please charge the \$40.00 assignment recordation fee to Deposit Account No. 23-1703.
- b. ( ) Enclosed is a check in the amount of \$40.
4. **Other document(s) or information included:**
- Copy of PCT/RO/101 - The PCT Request Form; and
  - Return postcard.

Respectfully submitted,

  
John M. Genova  
Reg. No. 32,224

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20 July 2000  
DATE

enclosures

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NEW COMPOUNDS

## FIELD OF THE INVENTION

5 The present invention relates to novel compounds, and pharmaceutically acceptable salts thereof, which inhibit basic carboxypeptidases, more specifically carboxypeptidase U, and thus can be used in the prevention and treatment of diseases wherein inhibition of carboxypeptidase U is beneficial. In further aspects, the invention relates to compounds of the invention for use in therapy; to processes for preparation of such new compounds; to  
10 pharmaceutical compositions containing at least one compound of the invention, or a pharmaceutically acceptable salt thereof, as active ingredient; and to the use of the active compounds in the manufacture of medicaments for the medical use indicated above.

## BACKGROUND OF THE INVENTION

15 Fibrinolysis is the result of a series of enzymatic reactions resulting in the degradation of fibrin by plasmin. The activation of plasminogen is the central process in fibrinolysis. The cleavage of plasminogen to produce plasmin is accomplished by the plasminogen activators, tissue-type plasminogen activator (t-PA) or urokinase-type plasminogen  
20 activator (u-PA). Initial plasmin degradation of fibrin generates carboxy-terminal lysine residues that serves as high affinity binding sites for plasminogen. Since plasminogen bound to fibrin is much more readily activated to plasmin than free plasminogen this mechanism provides a positive feedback regulation of fibrinolysis.

25 One of the endogenous inhibitors to fibrinolysis is carboxypeptidase U (CPU). CPU is also known as plasma carboxypeptidase B, active thrombin activatable fibrinolysis inhibitor (TAFIa), carboxypeptidase R and inducible carboxypeptidase activity. CPU is formed during coagulation and fibrinolysis from its precursor proCPU by the action of proteolytic enzymes *e.g.* thrombin, thrombin-thrombomodulin complex or plasmin. CPU cleaves basic  
30 amino acids at the carboxy-terminal of fibrin fragments. The loss of carboxy-terminal lysines and thereby of lysine binding sites for plasminogen then serves to inhibit fibrinolysis.

By inhibiting the loss of lysine binding sites for plasminogen and thus increase the rate of plasmin formation, effective inhibitors of carboxypeptidase U would be expected to facilitate fibrinolysis.

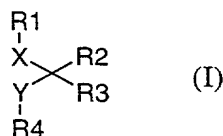
2-mercaptomethyl-3-guanidinoethylthiopropionic acid is reported as a carboxypeptidase N inhibitor. More recently, this compound has been shown to inhibit CPU, Hendriks, D. *et al.*, Biochimica et Biophysica Acta, 1034 (1990) 86-92.

Guanidinoethylmercaptosuccinic acid is reported as a carboxypeptidase N inhibitor. More recently, this compound has been shown to inhibit CPU, Eaton, D. L., *et al.*, The Journal of Biological Chemistry, 266 (1991) 21833-21838.

#### DISCLOSURE OF THE INVENTION

It has surprisingly been found that compounds of the Formula I are particularly effective as inhibitors of carboxypeptidase U and thereby useful as medicaments for the treatment or prophylaxis of conditions wherein inhibition of carboxypeptidase U is beneficial.

In one aspect, the invention thus relates to compounds of the general Formula I,



or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

R<sub>1</sub> represents,

C<sub>1</sub>-C<sub>6</sub> alkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O,

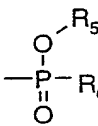
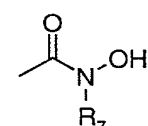
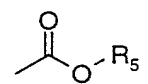
and substituted with one or more basic groups such as amino, amidino and/or guanidino;

or aryl, substituted with one or more basic groups such as amino, amidino and/or guanidino,

R<sub>2</sub> represents H, acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl,

aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, cyano, cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol, Z<sub>2</sub>N-CO-O-, ZO-CO-NZ- or Z<sub>2</sub>N-CO-NZ- group,

R<sub>3</sub> represents COOR<sub>5</sub>, SO(OR<sub>5</sub>), SO<sub>3</sub>R<sub>5</sub>, P=O(OR<sub>5</sub>)<sub>2</sub>, B(OR<sub>5</sub>)<sub>2</sub>, P=OR<sub>5</sub>(OR<sub>5</sub>), or tetrazole, or any carboxylic acid isostere,

R<sub>4</sub> represents a  -group, or a  -group, or a  -group,

R<sub>5</sub> represents H, C<sub>1</sub>-C<sub>6</sub> alkyl or aryl,

R<sub>6</sub> represents C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl, heterocyclyl, or an optionally N-substituted H<sub>2</sub>N-C(Z)-CONH-C(Z)- or H<sub>2</sub>N-C(Z)- group,

R<sub>7</sub> represents H or C<sub>1</sub>-C<sub>6</sub> alkyl,

X represents O, S, SO, SO<sub>2</sub>, C(Z)<sub>2</sub>, N(Z), NR<sub>7</sub>SO<sub>2</sub>, SO<sub>2</sub>NR<sub>7</sub>, NR<sub>7</sub>CO or CONR<sub>7</sub>,

Y represents O, N(Z), S, C(Z)<sub>2</sub>, or a single bond,

Z represents independently H, C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl or heterocyclyl,

with the proviso that when X represents O, S, SO, SO<sub>2</sub>, N(Z), NR<sub>7</sub>SO<sub>2</sub>, SO<sub>2</sub>NR<sub>7</sub>, or

NR<sub>7</sub>CO then Y represents C(Z)<sub>2</sub> or a single bond.

Preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt,



wherein

R<sub>1</sub> represents,

C<sub>1</sub>-C<sub>6</sub> alkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O,

and substituted with one or more basic groups such as amino, amidino and/or guanidino;

or aryl, substituted with one or more basic groups such as amino, amidino and/or guanidino,

R<sub>2</sub> represents H, acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl,

aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, cyano,

cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol, Z<sub>2</sub>N-CO-O-, ZO-CO-NZ- or Z<sub>2</sub>N-CO-NZ- group,

R<sub>3</sub> represents COOR<sub>5</sub>,

R<sub>4</sub> represents a  $\begin{array}{c} \text{O}-\text{R}_5 \\ | \\ \text{P}-\text{R}_6 \\ || \\ \text{O} \end{array}$  -group, or a  $\begin{array}{c} \text{O} \\ || \\ \text{C}-\text{N}-\text{OH} \\ | \\ \text{R}_7 \end{array}$  -group, or a  $\begin{array}{c} \text{O} \\ || \\ \text{C}-\text{O}-\text{R}_5 \end{array}$  -group,

R<sub>5</sub> represents H, C<sub>1</sub>-C<sub>6</sub> alkyl or aryl,

R<sub>6</sub> represents C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl, heterocyclyl, or an optionally N-substituted

H<sub>2</sub>N-C(Z)-CONH-C(Z)- or H<sub>2</sub>N-C(Z)- group,

R<sub>7</sub> represents H or C<sub>1</sub>-C<sub>6</sub> alkyl,

X represents C(Z)<sub>2</sub>,

Y represents O, N(Z), S, C(Z)<sub>2</sub>, or a single bond,

Z represents independently H, C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl or heterocyclyl.

More preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

$R_1$  represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O,

and substituted with one or more basic groups such as amino, amidino and/or guanidino;

$R_2$  represents H,  $C_1$ - $C_3$  alkyl, amino, halogen or hydroxy,

$R_3$  represents  $COOR_5$ ,

$R_4$  represents a  $\begin{array}{c} \text{O}-R_5 \\ | \\ -P- \\ || \\ \text{O} \end{array} R_6$  -group,

$R_5$  represents H,  $C_1$ - $C_6$  alkyl or aryl,

$R_6$  represents  $C_1$ - $C_6$  alkyl, aryl, cycloalkyl, heterocyclyl, or an optionally N-substituted  $H_2N-C(Z)-CONH-C(Z)-$  or  $H_2N-C(Z)-$  group,

X represents  $C(Z)_2$ ,

Y represents O or  $C(Z)_2$ ,

Z represents independently H or  $C_1$ - $C_6$  alkyl.


Other more preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

$R_1$  represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

R<sub>3</sub> represents COOR<sub>5</sub>,

R<sub>4</sub> represents a -group,

R<sub>7</sub> represents H or C<sub>1</sub>-C<sub>6</sub> alkyl,

Y represents  $C(Z)_2$  or a single bond,

Z represents independently H or C<sub>1</sub>-C<sub>6</sub> alkyl.

Yet other more preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt,

wherein

$R_1$  represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O,

and substituted with one or more basic groups such as amino, amidino and/or guanidino;

R<sub>2</sub> represents H, C<sub>1</sub>-C<sub>3</sub> alkyl, amino, halogen or hydroxy,

$R_3$  represents  $COOR_5$ ,

5     Z represents independently H or C<sub>1</sub>-C<sub>6</sub> alkyl.

10 R<sub>1</sub> represents,  
cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or  
guanidino;  
heterocyclyl, containing at least one nitrogen atom;

R<sub>2</sub> represents H, F, C<sub>1</sub> alkyl,

15 R<sub>3</sub> represents COOR<sub>5</sub>,

R<sub>5</sub> represents H, C<sub>1</sub>-C<sub>6</sub> alkyl or aryl,

R<sub>6</sub> represents C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl, heterocyclyl, or an optionally N-substituted

$$\text{H}_2\text{N}-\text{C}(\text{Z})-\text{CONH}-\text{C}(\text{Z})-\text{ or } \text{H}_2\text{N}-\text{C}(\text{Z})-\text{ group,}$$

20  $X$  represents  $C(Z)_2$ ,

Y represents O or C(Z)<sub>2</sub>.

Z represents independently H or C<sub>1</sub>-C<sub>6</sub> alkyl.

Other even more preferred compounds according to the present invention are those of  
 25 Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a  
 salt.

wherein

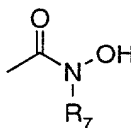
$R_1$  represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

5 heterocyclyl, containing at least one nitrogen atom;

$R_2$  represents H, F,  $C_1$  alkyl,

$R_3$  represents  $COOR_5$ ,

$R_4$  represents a  -group,

$R_5$  represents H,  $C_1$ - $C_6$  alkyl or aryl,

10  $R_7$  represents H or  $C_1$ - $C_6$  alkyl,

X represents  $C(Z)_2$ ,

Y represents  $C(Z)_2$  or a single bond,

Z represents independently H or  $C_1$ - $C_6$  alkyl.

15 Yet other even more preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt,

wherein

$R_1$  represents,

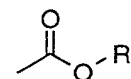
20 cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

$R_2$  represents H, F,  $C_1$  alkyl,

$R_3$  represents  $COOR_5$ ,

25

$R_4$  represents a  -group,

$R_5$  represents H,  $C_1$ - $C_6$  alkyl or aryl,

X represents  $C(Z)_2$ ,

Y represents  $C(Z)_2$  or a single bond,

Z represents independently H or  $C_1$ - $C_6$  alkyl.

5

Most preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

$R_1$  represents pyridyl or piperidinyl,

10  $R_2$  represents H,

$R_3$  represents  $COOR_5$ ,

$R_4$  represents a  $\begin{array}{c} \text{O}-R_5 \\ | \\ -P- \\ || \\ \text{O} \end{array} R_6$  -group,

$R_5$  represents H,

$R_6$  represents  $C_1$ - $C_6$  alkyl or an optionally N-substituted  $H_2N-C(Z)-CONH-C(Z)-$  or  $H_2N-$

15  $C(Z)-$  group,

X represents  $CHZ$ ,

Y represents  $CHZ$ ,

Z represents independently H or  $C_1$ - $C_6$  alkyl.

20 Other most preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

$R_1$  represents pyridyl or piperidinyl,

$R_2$  represents H,

25  $R_3$  represents  $COOR_5$ ,

$R_4$  represents a  $\begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{N}-\text{OH} \\ | \\ R_7 \end{array}$  -group,

R<sub>5</sub> represents H,

R<sub>7</sub> represents H,

X represents CHZ,

5 Y represents CHZ or a single bond,

Z represents independently H or C<sub>1</sub>-C<sub>6</sub> alkyl.

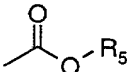
Yet other most preferred compounds according to the present invention are those of  
Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a  
10 salt,

wherein

R<sub>1</sub> represents pyridyl or piperidinyl,

R<sub>2</sub> represents H,

R<sub>3</sub> represents COOR<sub>5</sub>,

R<sub>4</sub> represents a  -group,

15 R<sub>5</sub> represents H,

X represents CHZ,

Y represents CHZ or a single bond,

Z represents independently H or C<sub>1</sub>-C<sub>6</sub> alkyl.

20 The following definitions shall apply throughout the specification and the appended claims:

The term "basic group" denotes a basic group, wherein the conjugate acid of said basic  
25 group has a pK<sub>a</sub> of from about -5 to about 25, preferably of from 1 to 15.

The term "carboxylic acid isostere" denotes an acidic group having a pK<sub>a</sub> of from about -5  
to about 25, preferably of from 1 to 15.

The term "C<sub>1</sub>-C<sub>6</sub> alkyl" denotes a straight or branched, saturated or unsaturated, substituted or unsubstituted alkyl group having 1 to 6 carbon atoms in the chain wherein the alkyl group may optionally be interrupted by one or more heteroatoms selected from O, N or S. Examples of said alkyl include, but is not limited to, methyl, ethyl, ethenyl, ethynyl, n-propyl, iso-propyl, propenyl, iso-propenyl, propynyl, n-butyl, iso-butyl, sec-butyl, t-butyl, butenyl, iso-butenyl, butynyl and straight- and branched-chain pentyl and hexyl.

The term "C<sub>1</sub>-C<sub>3</sub> alkyl" denotes a straight or branched, saturated or unsaturated, substituted or unsubstituted alkyl group having 1 to 3 carbon atoms in the chain wherein the alkyl group may optionally be interrupted by one or more heteroatoms selected from O, N or S. Examples of said alkyl include, but is not limited to, methyl, ethyl, ethenyl, ethynyl, n-propyl, iso-propyl, propenyl, iso-propenyl, propynyl.

The term "C<sub>1</sub> alkyl" denotes a substituted or unsubstituted alkyl group having 1 carbon atom. An example of said alkyl include, but is not limited to, methyl,

The term "C<sub>1</sub>-C<sub>6</sub> alkoxy" denotes an alkyl-O-group, wherein C<sub>1</sub>-C<sub>6</sub> alkyl is as defined above.

The term "C<sub>1</sub>-C<sub>3</sub> alkoxy" denotes an alkyl-O-group, wherein C<sub>1</sub>-C<sub>3</sub> alkyl is as defined above.

The term "heterocyclyl" denotes a substituted or unsubstituted, 4- to 10- membered monocyclic or multicyclic ring system in which one or more of the atoms in the ring or rings is an element other than carbon, for example nitrogen, oxygen or sulfur, especially 4-, 5- or 6-membered aromatic or alifatic heterocyclic groups, and includes, but is not limited to azetidine, furan, thiophene, pyrrole, pyrroline, pyrrolidine, dioxolane, oxathiolane, oxazolane, oxazole, thiazole, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, isoxazole, isothiazole, oxadiazole, furazan, triazole, thiadiazole, pyran, pyridine, piperidine, dioxane, morpholine, dithiane, oxathiane, thiomorpholine, pyridazine, pyrimidine, pyrazine, piperazine, triazine, thiadiazine, dithiazine, azaindole, azaindoline,



indole, indoline, naphthyridine groups, and shall be understood to include all isomers of the above identified groups. The term "azetidiny" shall for example be understood to include the 2-, and 3-isomers and the terms "pyridyl" and "piperidiny" shall for example be understood to include the 2-, 3-, and 4-isomers.

5

The term "cycloalkyl" denotes a saturated or unsaturated, substituted or unsubstituted, non-aromatic ring composed of 3, 4, 5, 6 or 7 carbon atoms, and includes, but is not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclopentadienyl, cyclohexadienyl and cycloheptadienyl groups,

10

The term "halogen" includes fluoro, chloro, bromo and iodo groups.

The term "aryl" denotes a substituted or unsubstituted C<sub>6</sub>-C<sub>14</sub> aromatic hydrocarbon and includes, but is not limited to, phenyl, naphthyl, indenyl, anthracenyl, fenantrenyl, and fluorenyl.

15

The term "aryloxy" denotes an aryl-O-group, wherein aryl is as defined above.

The term "acyl" denotes an alkyl-CO-group, wherein alkyl is as defined above.

20

The term "aroyl" denotes an aryl-CO-group, wherein aryl is as defined above.

The term "alkylthio" denotes an alkyl-S-group, wherein alkyl is as defined above.

25

The term "arylthio" denotes an aryl-S-group, wherein aryl is as defined above.

The term "aroylamino" denotes an aroyl-N(Z)-group, wherein aroyl and Z is as defined above.

30

The term "acylamino" denotes an acyl-N(Z)-group, wherein acyl and Z is as defined above.

The term "carbamoyl" denotes a  $\text{H}_2\text{N-CO-}$ group.

The term "alkylcarbamoyl" denotes a  $\text{Z}_2\text{N-CO-}$ group wherein Z is as defined above.

5 The term "substituted" denotes an " $\text{C}_1$  alkyl", " $\text{C}_1\text{-C}_3$  alkyl", " $\text{C}_1\text{-C}_6$  alkyl", "cycloalkyl", "heterocyclyl", "aryl",  $\text{H}_2\text{N-C(Z)-CONH-C(Z)-}$  or a  $\text{H}_2\text{N-C(Z)-}$  group as defined above which is substituted by one or more acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl, aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, 10 cyano, cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol, thio,  $\text{Z}_2\text{N-CO-O-}$ ,  $\text{ZO-CO-NZ-}$ , or  $\text{Z}_2\text{N-CO-NZ-}$  groups.

Both the pure enantiomers, racemic mixtures and unequal mixtures of two enantiomers are within the scope of the present invention. It should also be understood that all the 15 diastereomeric forms possible are within the scope of the invention. Also included in the invention are derivatives of the compounds of the Formula I which have the biological function of the compounds of Formula I, such as prodrugs.

Depending on the process conditions the compounds of Formula I are obtained either in 20 neutral or salt form or as a solvate, e.g. a hydrate, and are all within the scope of the present invention.

### *Preparation*

25 The present invention also provides the process A-C for the manufacture of compounds with the general Formula I.

### Process A

Process A for manufacture of compounds with the general Formula I, wherein  $\text{R}_1$ ,  $\text{R}_5$ ,  $\text{R}_6$ , 30 and Z are as defined above and  $\text{R}_2$  is H,  $\text{R}_3$  is  $\text{COOR}_5$ ,

R<sub>4</sub> represents a  $\begin{array}{c} \text{O}-\text{R}_5 \\ | \\ -\text{P}-\text{R}_6 \\ || \\ \text{O} \end{array}$  -group,

X is C(Z)<sub>2</sub>, Y is C(Z)<sub>2</sub> and comprises the following steps:

a) Compounds of the general Formula II,

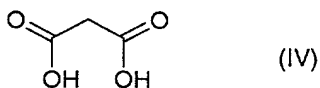


wherein R<sub>1</sub> and Z is as defined for Formula I and X is C(Z)<sub>2</sub>, which are either commercially available or are available using known techniques, can be converted into a compound of the general Formula III,

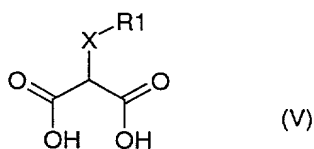


wherein L is a suitable leaving group, such as a chloro, bromo, iodo, triflate or tosyl group, under standard conditions using a suitable reagent, such as PPh<sub>3</sub>/CBr<sub>4</sub>, TosCl/pyridine or (CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>O/TEA).

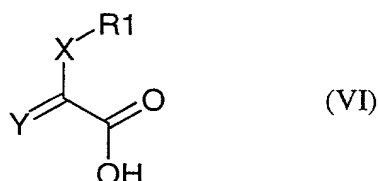
b) Compounds of the general Formula III can thereafter be reacted with compounds of the general Formula IV,



which are either commercially available or are available using known technics, in the presence of a suitable base, such as K<sub>2</sub>CO<sub>3</sub> or NaH, under standard conditions to give compounds of the general Formula V,

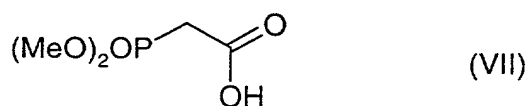


c) Compounds of the general Formula V can thereafter be converted to compounds of the general Formula VI,



5 by treatment with formaldehyd in the presence of a suitable base, such as  $\text{Et}_2\text{NH}$ , under standard conditions.

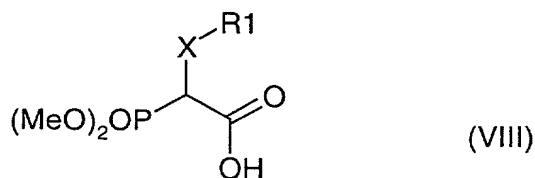
However, if Y is  $\text{CH}(\text{Z})$  then compounds of the general Formula VI can be prepared by treating compounds of the general Formula VII,



with an alkylating agent of the general Formula III,



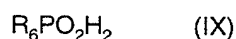
15 wherein  $\text{R}_1$  is as defined for Formula I and L is a suitable leaving group, such as a chloro, bromo, iodo, triflate or tosyl group, in the presence of a suitable base, such as LDA or NaH, under standard conditions to give compounds of the general Formula VIII,



Compounds of the general Formula VIII can thereafter be reacted with an appropriate aldehyde CHO(Z), wherein Z is as defined for Formula I, in the presence of a suitable base, such as K<sub>2</sub>OtBu, LDA or NaH, under standard conditions to give to give a compound of the general Formula VI.

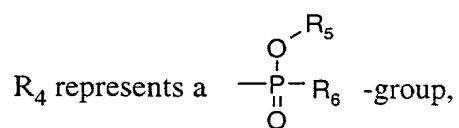
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d) Compounds of the general Formula VI can be further reacted with compounds of the general Formula IX



10

wherein R<sub>6</sub> is as defined for Formula I, in the presnec of a suitable reagent, such as BSA or HMDS, under standard conditions to give compounds of the general Formula I, wherein R<sub>1</sub>, R<sub>5</sub>, R<sub>6</sub> and Z are as defined above, R<sub>2</sub> is H, R<sub>3</sub> is COOR<sub>5</sub>,

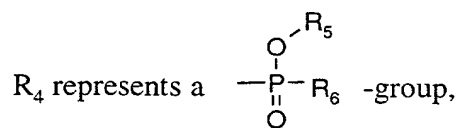


X is C(Z)<sub>2</sub>, and Y is C(Z)<sub>2</sub>.

15

### Process B

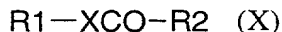
Process B for manufacture of compounds with the general Formula I, wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>5</sub>, R<sub>6</sub>, and Z are as defined above, R<sub>3</sub> is COOR<sub>5</sub>, X is C(Z)<sub>2</sub>, Y is O, and



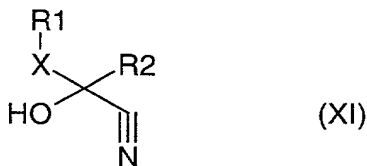
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comprises the following steps:

a) Reacting a compound of the general Formula X,

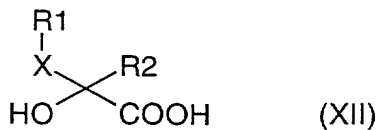


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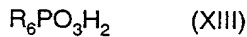
wherein  $R_1$  and  $R_2$  are as defined in Formula I and X is  $C(Z)_2$ .

10



15 wherein  $R_1$  and  $R_2$  are as defined in Formula I and X is  $C(Z)_2$ .

c) Compounds of the general Formula XII can thereafter be reacted with compounds of the general Formula XIII,



20

conditions to give compounds of the general Formula I, wherein  $R_1$ ,  $R_2$ ,  $R_5$ ,  $R_6$  and  $Z$  are as defined above,  $R_3$  is  $\text{COOR}_5$ ,

$R_4$  represents a  $\begin{array}{c} \text{O}-R_5 \\ | \\ -\text{P}-R_6 \\ || \\ \text{O} \end{array}$  -group,

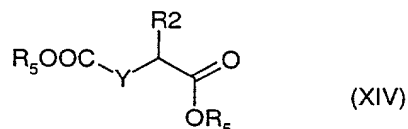
$X$  is  $\text{C}(Z)_2$  and  $Y$  is  $\text{O}$ .

5

### Process C

Process C for manufacture of compounds with the general Formula I, wherein  $R_1$  and  $R_2$  are as defined above and  $X$  and  $Y$  is  $\text{C}(Z)_2$  or a single bond and  $R_3$  and  $R_4$  are  $\text{COOR}_5$ ,  
 10 comprises the following steps,

a) reacting a compound of the general Formula XIV,

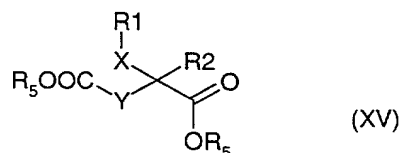


15 wherein  $R_2$  and  $R_5$  are as defined in Formula I and  $Y$  is  $\text{C}(Z)_2$  or a single bond, which are either commercially available, well known in the literature, or are available using known techniques, with a compound of the general Formula III,



wherein  $R_1$  is as defined for Formula I,  $X$  is  $\text{C}(Z)_2$  and  $L$  is a suitable leaving group, such as  $\text{Cl}$ ,  $\text{Br}$ ,  $\text{I}$  or tosyl, in the presence of a suitable base, such as  $\text{LDA}$  or  $\text{NaH}$  under standard  
 20 conditions, to give a compound of the general Formula XV,

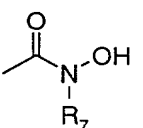
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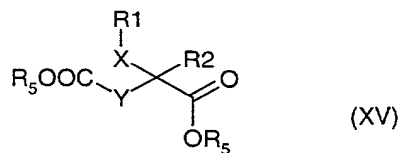
b) hydrolysing a compound of the general Formula XV, for example by treatment with aqueous NaOH or aqueous TFA under standard conditions to give compounds of the general Formula I, wherein R<sub>1</sub> and R<sub>2</sub> are as defined above and X and Y is C(Z)<sub>2</sub> or a single bond and R<sub>3</sub> and R<sub>4</sub> are COOH.

#### Process D

Process D for manufacture of compounds with the general Formula I, wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>5</sub>, R<sub>7</sub>, X, Y and Z are as defined above, R<sub>3</sub> is COOR<sub>5</sub> and

R<sub>4</sub> represents a  -group, comprises the following steps:

a) Compounds of the general Formula XV,



can be reacted with compounds of the general Formula XVI,





5

$R_4$  represents a

10

15

20

25

The use of protecting groups is described in 'Protective Groups in Organic Synthesis', 2nd edition, T.W. Greene & P.G.M. Wutz, Wiley-Interscience (1991). The protective group may also be a polymer resin such as Wang resin or a 2-chlorotrityl chloride resin.

5 It will also be appreciated by those skilled in the art, although such protected derivatives of compounds of Formula I may not possess pharmacological activity as such, they may be administered parenterally or orally and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". All prodrugs of compounds of Formula I are included  
10 within the scope of the invention.

It should also be understood that all polymorphs, amorphous forms, anhydrides, hydrates, solvates of the compounds of the present invention are within the scope of the invention.

15 *Pharmaceutical formulations*

In yet a further aspect, the invention relates to pharmaceutical compositions containing at least one compound of the present invention, or a pharmaceutically acceptable salt thereof, as active ingredient.

20

For clinical use, the compounds of the invention are formulated into pharmaceutical formulations for oral, intravenous, subcutaneous, tracheal, bronchial, intranasal, pulmonary, transdermal, buccal, rectal, parenteral or other mode of administration. The pharmaceutical formulation contains a compound of the invention in combination with one  
25 or more pharmaceutically acceptable ingredients. The carrier may be in the form of a solid, semi-solid or liquid diluent, or a capsule. These pharmaceutical preparations are a further object of the invention. Usually the amount of active compounds is between 0.1-95% by weight of the preparation.

30 In the preparation of pharmaceutical formulations containing a compound of the present invention the compound selected may be mixed with solid, powdered ingredients, such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin,

or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture may then be processed into granules or pressed into tablets.

5 Soft gelatine capsules may be prepared with capsules containing a mixture of the active compound or compounds of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Hard gelatine capsules may contain granules of the active compound. Hard gelatine capsules may also contain the active compound in combination with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato  
10 starch, corn starch, amylopectin, cellulose derivatives or gelatine.

Dosage units for rectal administration may be prepared (i) in the form of suppositories which contain the active substance mixed with a neutral fat base; (ii) in the form of a gelatine rectal capsule which contains the active substance in a mixture with a vegetable  
15 oil, paraffin oil or other suitable vehicle for gelatine rectal capsules; (iii) in the form of a ready-made micro enema; or (iv) in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

Liquid preparations may be prepared in the form of syrups or suspensions, e.g. solutions or  
20 suspensions containing the active ingredient and the remainder consisting, for example, of sugar or sugar alcohols and a mixture of ethanol, water, glycerol, propylene glycol and polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, preservatives, saccharine and carboxymethyl cellulose or other thickening agents. Liquid preparations may also be prepared in the form of a dry powder to  
25 be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administration may be prepared as a solution of a compound of the invention in a pharmaceutically acceptable solvent. These solutions may also contain stabilizing ingredients and/or buffering ingredients. Solutions for parenteral administration  
30 may also be prepared as a dry preparation to be reconstituted with a suitable solvent before use.

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The typical daily dose of the active substance varies within a wide range and will depend on various factors such as for example the individual requirement of each patient, the route of administration and the disease. In general, oral and parenteral dosages will be in the range of 0.1 to 1000 mg per day of active substance.

5

*Medical and pharmaceutical use*

The compounds of the invention are inhibitors of carboxypeptidase U either as such or, in the case of prodrugs, after administration. The compounds of the invention are thus  
10 expected to be useful in those conditions where inhibition of carboxypeptidase U is beneficial, such as in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of mammals, including man.

It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions  
15 associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include protein C resistance and inherited or acquired deficiencies in antithrombin III, protein C, protein S and heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulatory and septic shock, circulating antiphospholipid antibodies, homocysteinemia, heparin induced  
20 thrombocytopenia and defects in fibrinolysis. The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions. The compounds of the invention are further indicated in the treatment of conditions where there is an undesirable excess of proCPU/CPU.

25 Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism, arterial thrombosis (e.g. in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction.

30

Moreover, the compounds of the invention are expected to have utility in prophylaxis of re-occlusion and restenosis (*i.e.* thrombosis) after thrombolysis, percutaneous trans-luminal

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angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism, fibrinolytic treatment when blood is in contact with foreign surfaces in the body, such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device, and fibrinolytic treatment when blood is in contact with medical devices outside the body, such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

The compounds of the invention may also be combined and/or coadministered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor ( $P_2T$ ) antagonists and thrombin inhibitors.

The compounds of the invention may further be combined and/or coadministered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction and stroke.

#### *In vitro experiments*

The inhibiting effect of the compounds of the present invention was estimated using the assay described in: Dirk Hendriks, Simon Scharpé and Marc van Sande, Clinical Chemistry, 31, 1936-1939 (1985); and Wei Wang, Dirk F. Hendriks, Simon S. Scharpé, The Journal of Biological Chemistry, 269, 15937-15944 (1994).

## EXAMPLES

General Experimental Procedures

5 Mass spectra were recorded on a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer equipped with an electrospray interface (FAB-MS) and VG Platform II mass spectrometer equipped with an electrospray interface (LC-MS).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR measurements were performed on Varian UNITY plus 400, 500 and 600 spectrometers, operating at  $^1\text{H}$  frequencies of 400, 500 and 600 MHz respectively. Chemical shifts are  
10 given in ppm with the solvent as internal standard. Organic extracts were dried using  $\text{MgSO}_4$  or  $\text{Na}_2\text{SO}_4$  as the drying agent. Chromatography separations were performed using Merck Silica gel 60 (0.063-0.200 mm). HPLC separations were performed on a HIGHCROM KR100-10C8 column.

15 Example 15-Amino-2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-pentanoic acid(a) 5-tert-Butoxycarbonylamino-2-hydroxy-pentanoic acid

20 Di-*t*-butyl dicarbonate (30.8 g, 0.141 mol) was added in portions during 5 min to a solution of 5-amino-2-hydroxy-pentanoic acid (17.0 g, 0.128 mol) in 0.5 M NaOH (240 mL) and dioxan (240 mL) at 5°C. The mixture was stirred for 2.5 h at room temperature. During this time 0.5 M NaOH was added to maintain pH 9-10. The dioxan was removed under reduced pressure and the aqueous phase was washed with diethyl ether. The aqueous phase was  
25 acidified to pH 2-3 with  $\text{KHSO}_4$  and extracted with ethyl acetate (3 x 300 mL). The pooled organic phases was washed with water and brine, dried and concentrated under reduced pressure to give crude 5-tert-butoxycarbonylamino-2-hydroxy-pentanoic acid (22.0 g, 73.7%).

30 (b) 5-tert-Butoxycarbonylamino-2-hydroxy-pentanoic acid methyl ester

A solution of methyl iodide (11.5 mL, 0.189 mol) in DMF (50 mL) was added dropwise during 15 min. to a mixture of 5-tert-butoxycarbonylamino-2-hydroxy-pentanoic acid

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(22.0 g, 94.4 mmol) and  $\text{NaHCO}_3$  (11.8 g, 141 mmol) in DMF (150 mL). After stirring over night, water was added and the mixture was extracted with ethyl acetate. The pooled organic phases were washed with water and brine, dried and concentrated under reduced pressure. The crude product was purified using chromatography (heptane/ethyl acetate, 1:1) to give 5-*tert*-butoxycarbonylamino-2-hydroxy-pentanoic acid methyl ester 9.9 g, 42.5 %)

(c) 2-[(1-Benzyloxycarbonylamino-2-methyl-propyl)-methoxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid methyl ester

A solution of PyBOP (2.1 g, 4.0 mmol) in DMF (3 mL) was added to a mixture of (1-benzyloxycarbonylamino-2-methyl-propyl)-phosphonic acid monomethyl ester (1.0 g, 3.32 mmol) and 5-*tert*-butoxycarbonylamino-2-hydroxy-pentanoic acid methyl ester (865 mg, 3.5 mmol) in DMF (4 mL) under argon. DIPEA (2.28 mL, 13.3 mmol) was added dropwise and the mixture was stirred over night. Ethyl acetate was added and the mixture was washed with 10 %  $\text{KHSO}_4$ , satd.  $\text{NaHCO}_3$  and brine and dried. Concentration under reduced pressure followed by chromatography (heptane/EtOAc, 1:1  $\rightarrow$  1:6) gave 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-methoxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid methyl ester (1.21 g, 69 %).

(d) 2-[(1-Benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid

1 M LiOH (5 mL) was added to a solution of 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-methoxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid methyl ester (187 mg, 0.35 mmol) in acetonitrile (5 mL). The mixture was stirred at 50°C over night and concentrated under reduced pressure. The crude product was purified using chromatography (iPrOH/conc. aq.  $\text{NH}_3/\text{H}_2\text{O}$ , 4:2:1) to yield 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid (180 mg, 100 %).

(e) 5-Amino-2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-pentanoic acid

TFA (3 mL) was added to a solution of 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid (150 mg, 0.3 mmol) in methylene chloride/acetonitrile (1:1, 15 mL). The solution was stirred for 120 min and concentrated under reduced pressure to give the title compound as the TFA salt (174 mg, 100 %).

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 1.02 (t, 6H), 1.66-2.0 (m, 4H), 2.23 (m, 1H), 2.93 (m, 2H), 3.91 (m, 1H), 4.85 (bs, 1H), 5.12 (m, 2H), 7.28-7.42 (m, 5H).

MS (+) 403.3 (M+1).

### Example 2

2-[(1-Benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-5-guanidino-pentanoic acid

(a) 2-[(1-Benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-5-guanidino-pentanoic acid

A solution of S-methylisothiurea hydrogen sulfate (25 mg, 90 μmol) in 1 M NaOH (0.18 mL) was added to a solution of 5-amino-2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyloxy]-pentanoic acid (36 mg, 90 μmol) and 1 M NaOH (0.18 mL) in water/MeOH (1:1, 0.4 mL). The reaction mixture was stirred at 50°C for 6 h and

concentrated under reduced pressure. The crude product was purified using HPLC (0-50 % acetonitrile, 0.1% TFA in water) to give the title compound as the TFA salt (19 mg, 38 %)

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 1.02 (t, 6H), 1.60-1.98 (m, 4H), 2.23 (m, 1H), 3.20 (m, 2H), 3.91 (m, 1H), 4.82 (bs, 1H), 5.11 (m, 2H), 7.26-7.42 (m, 5H).

MS (+) 445 (M+1).

### Example 3

5-Amino-2-[[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy]-pentanoic acid

(a) 2-[[1-(2-Benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy]-5-*tert*-butoxycarbonylamino-pentanoic acid methyl ester



Thionyl chloride (49  $\mu$ L, 0.67 mmol) was added dropwise to a solution of [1-(2-benzyloxy-carbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-phosphonic acid (208 mg, 0.48 mmol) in DMF (5 mL) at  $-20^{\circ}\text{C}$  under argon. The mixture was stirred for 35 min at  $-5^{\circ}\text{C}$ . A solution of 5-*tert*-Butoxycarbonylamino-2-hydroxy-pentanoic acid methyl ester (166 mg, 0.67 mmol) in DMF (1 mL) was added and the mixture was stirred for 90 min at room temperature. Ethyl acetate was added and the mixture was washed with 1 M HCl, dried and concentrated under reduced pressure. The crude product was purified using chromatography ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ , 10:1:0  $\rightarrow$  10:5:1) to give 2-{[1-(2-benzyloxy-carbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-*tert*-butoxycarbonylamino-pentanoic acid methyl ester (211 mg, 66 %).

(b) 2-{[1-(2-Benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-*tert*-butoxycarbonylamino-pentanoic acid

1 M LiOH (3.5 mL) was added to a solution of 2-{[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-*tert*-butoxycarbonylamino-pentanoic acid methyl ester (211 mg, 0.32 mmol) in acetonitrile (3.5 mL) and the mixture was stirred for 3 h. Ethyl acetate was added and the mixture was washed with 1 M HCl, dried and concentrated to give crude 2-{[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-*tert*-butoxycarbonylamino-pentanoic acid (208 mg, 100 %).

(c) 5-Amino-2-{[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-pentanoic acid

TFA (5 mL) was added to a solution of 2-{[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-*tert*-butoxycarbonylamino-pentanoic acid (208 mg, 0.32 mmol) in acetonitrile (5 mL) and the mixture was stirred for 90 min. The reaction mixture was concentrated under reduced pressure to give the crude title compound as the TFA salt (212 mg, 100 %). 20 mg of the crude title compound was purified using chromatography (*i*PrOH/conc. aq.  $\text{NH}_3/\text{H}_2\text{O}$ , 4:2:1) to give the title compound as the TFA salt (19 mg, 94 %).

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 0.85-0.95 (m, 6H), 1.70-2.0 (m, 4H), 2.05-2.13 (m, 1H), 2.85-3.05 (m, 2H), 3.05-3.12 (m, 1H), 4.10 (bs, 1H), 4.55 (m, 1H), 4.90 (m, 1H), 5.09 (s, 2H), 7.20-7.35 (m, 10H).

5 Example 4

2-([1-(2-Benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy)-5-guanidino-pentanoic acid

(a) 5-Amino-2-hydroxy-pentanoic acid methyl ester

10 TFA (2 mL) was added to a solution of 5-*tert*-butoxycarbonylamino-2-hydroxy-pentanoic acid methyl ester in methylene chloride (10 mL) and the mixture was stirred for 3 h and then concentrated under reduced pressure to give crude 5-amino-2-hydroxy-pentanoic acid methyl ester (1 g).

15 (b) 5-(Guanidino-ω, ω'-bis(*tert*-Butoxycarbonyl)-2-hydroxy-pentanoic acid methyl ester

To a solution of 5-amino-2-hydroxy-pentanoic acid methyl ester (0.5 g, 2.0 mmol) in acetonitrile (5 mL) was added *tert*-butoxycarbonylimino-pyrazol-1-yl-methyl)-carbamic acid *tert*-butyl ester (0.77g, 2.5 mmol) followed by DIPEA (0.86 mL, 5 mmol). After stirring for 60 min ethyl acetate was added. The mixture was washed with 1 M HCl, satd.

20 NaHCO<sub>3</sub> and brine, dried and concentrated under reduced pressure. The crude product was purified using chromatography (heptane/ethyl acetate, 1:0 → 1:3) to give 5-(guanidino-ω, ω'-bis(*tert*-butoxycarbonyl)-2-hydroxy-pentanoic acid methyl ester (0.27 g, 35 %).

(c) 2-([1-(2-Benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-

25 hydroxy-phosphinoyloxy)-5-[guanidino-ω, ω'-bis(*tert*-Butoxycarbonyl)]-pentanoic acid methyl ester

Thionyl chloride (70 μL, 0.97 mmol) was added dropwise to a solution of [1-(2-benzyloxy-carbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-phosphonic acid (301 mg,

30 0.69 mmol) in DMF (5 mL) at -20°C under argon. The mixture was stirred for 20 min at

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-5°C. A solution of 5-(guanidino- $\omega$ ,  $\omega'$ -bis(*tert*-Butoxycarbonyl)-2-hydroxy-pentanoic acid methyl ester (270 mg, 0.69 mmol) in DMF (1 mL) was added and the mixture was stirred for 180 min at room temperature. Ethyl acetate was added and the mixture was washed with 1 M HCl, dried and concentrated under reduced pressure. The crude product was purified using chromatography (toluene/ethyl acetate, 1:1  $\rightarrow$  0:1) to give 2-{{1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-[guanidino- $\omega$ ,  $\omega'$ -bis(*tert*-butoxycarbonyl)]-pentanoic acid methyl ester (0.27 g, 48 %).

(d) 2-{[1-(2-Benzoyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-[guanidino- $\omega$ ,  $\omega'$ -bis(*tert*-Butoxycarbonyl)]-pentanoic acid

A solution of LiOH (42 mg, 1.0 mmol) in water (1.0 mL) was added to a solution of 2-{{[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-[guanidino- $\omega$ ,  $\omega'$ -bis(*tert*-butoxycarbonyl)]-pentanoic acid methyl ester (160 mg, 0.2 mmol) in acetonitrile (1.0 mL) and the mixture was stirred for 15 min. Ethyl acetate was added and the mixture was washed with 1 M HCl and brine, dried and concentrated to give crude 2-{{[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy}-5-[guanidino- $\omega$ ,  $\omega'$ -bis(*tert*-butoxycarbonyl)]-pentanoic acid (160 mg, 100 %).

(e) 2-{[1-(2-Benzoyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinyloxy}-5-guanidino-pentanoic acid

TFA (2 mL) was added to a solution of 2-[[1-(2-benzyloxycarbonylamino-3-phenyl-propionylamino)-2-methyl-propyl]-hydroxy-phosphinoyloxy]-5-[guanidino- $\omega$ ,  $\omega'$ -bis(*tert*-butoxycarbonyl)]-pentanoic acid (160 mg, 0.2 mmol) in acetonitrile (5 mL) and the mixture was stirred for 60 min and then concentrated under reduced pressure. The crude product was purified using chromatography (iPrOH/conc. aq.  $\text{NH}_3/\text{H}_2\text{O}$ , 4:2:1) to give the title compound as the TFA salt (30 mg, 21 %).

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 0.80-0.98 (m, 6H), 1.53-1.95 (m, 4H), 2.01-2.30 (m, 1H), 2.90 (m, 1H), 3.10-3.30 (m, 3H), 3.94-4.10 (m, 1H), 4.41-4.55 (m, 1H), 4.68 (m, 1H), 5.03 (m, 2H), 7.18-7.37 (m, 5H).

MS (+) 592 (M+1).

### Example 5

#### 2-Hydroxycarbamoyl-4-piperidin-4-yl-butyric acid

5

##### (a) Piperidin-4-yl-acetic acid

Pyridin-4-yl-acetic acid hydrochloride (20.0 g, 115 mmol) was added to water/25 % ammonia (125 mL:10mL). The mixture was degassed and flushed with nitrogen before addition of rhodium on activated alumina (0.45 g). The mixture was again degassed, then stirred in a hydrogen atmosphere at 50 bar for 16 h. Filtration of the reaction mixture through filter paper afforded the bulk of the catalyst which was recycled after washing with methanol. The filtrate was then filtered through Celite and concentrated to afford a white solid (19.7 g, 96 % yield).

##### (b) 4-Carboxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester

To a solution of piperidin-4-yl-acetic acid (19.7 g, 148 mmol) in THF-water (417 mL, 1:1) was added di-*tert*-butyl dicarbonate (32.3 g, 148 mmol) and sodium bicarbonate (12.5 g, 148 mmol), and the reaction stirred at room temperature for 16 h. THF was then removed under reduced pressure and the aqueous phase extracted with dichloromethane and the organic layer discarded. The aqueous layer was then acidified to pH 1-2 with 1 M HCl solution and extracted with ethyl acetate. The organic phase was washed with brine, dried and concentrated under reduced pressure to give 4-carboxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (16.7 g, 46 %).

##### (c) 4-(2-Hydroxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester

To a solution of 4-carboxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (16.7 g, 69.0 mmol) in THF (100 mL) was added diborane (151 mL, 1.0 M solution in THF) over a period of 10 min at 0°C. Hydrogen gas was rapidly evolved and after gas evolution had ceased the reaction was stirred at room temperature for 1 h. The reaction mixture was again cooled to 0°C, and 1 M aqueous HCl was added dropwise to the reaction mixture with further evolution of hydrogen. Addition of HCl was continued until the evolution of hydrogen had almost ceased. The mixture was then stirred for 10 min and made basic (pH

13-14) by the addition of 1 M NaOH solution. The aqueous solution was extracted with ethyl acetate, the organic phase washed with brine, dried and concentrated under reduced pressure to yield 4-(2-hydroxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (15.2 g, 97 %).

5 (d) 4-(2-Oxo-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester

Periodinane (36.1 g, 85.2 mmol) was added to 4-(2-hydroxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (15.0 g, 65.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (230 mL) and stirred for 90 min.

Diethyl ether (560 ml) was added and precipitates were removed by extraction with 10 %  
10 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/saturated NaHCO<sub>3</sub> (1:1, 350 mL). The organic layer was washed with 0.5 M NaOH solution and brine. The organic phase was dried and concentrated under reduced pressure to yield 4-(2-Oxo-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (8.50 g, 57 %).

15 (e) 4-[2-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester.

To a solution of meldrums acid (1.68 g, 11.66 mmol) and 4-(2-oxo-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.21 g, 9.72 mmol) in dichloromethane (40 mL) was added acetic acid (0.055 mL, 0.972 mmol) and piperidine (0.096 mL, 0.972 mmol). The mixture  
20 was heated at reflux for 3 h, and then allowed to attain room temperature. After being diluted with *tert*-butyl methyl ether, the mixture was washed with NaHCO<sub>3</sub> (sat.) and brine. The organic phase was dried, filtered and concentrated. The residue was dissolved in a mixture of EtOH (40 mL) and acetic acid (20 mL). The solution was cooled to 0°C, and NaBH<sub>4</sub> (0.554 g, 14.6 mmol) was added in portions after which the solution was allowed to  
25 stir for 30 min at rt and then acidified to pH 3 with 1 M HCl. The solution was extracted several times with dichloromethane. The combined organic phases were dried, filtered, concentrated and filtered through a pad of silica gel (dichloromethane). The solvent was evaporated to give 4-[2-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester as a colorless oil (2.30 g, 65 %) which solidified on  
30 standing.

(f) 2-Hydroxycarbamoyl-4-piperidin-4-yl-butyric acid.

A GC-autosample vial (2 mL) equipped with a septum cap and a small stirbar was charged with 4-[2-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (17.8 mg, 0.05 mmol) and flushed with nitrogen. N,O-Bis(trimethylsilyl)-hydroxylamine (0.2 mL) was added via syringe and the resulting solution was stirred at rt over night. The mixture was concentrated under vacuum and the residue dissolved in dichloromethane/MeOH (4:1) and applied onto a small plug of ion exchange resin (200 mg, isolate™, aminoresin), washed with dichloromethane/MeOH (4:1) and then eluted with dichloromethane/MeOH/AcOH (3:1:1). The eluate was concentrated, the residue dissolved in dichloromethane/TFA (1:1, 2 mL) and stirred for 1h at room temperature. Evaporation of the solvent gave the title compound as the TFA salt (16 mg, 93 %) as a colourless oil.

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 1.21-1.40 (m, 4H), 1.53-1.62 (m, 1H), 1.80-1.99 (m, 4H), 2.90-2.98 (m, 2H), 3.06 (t, 1H), 3.32-3.39 (m, 2H).  
M (+) 231 (M+1).

#### Example 6

##### N-hydroxy-2-piperidin-3-ylmethyl-malonamic acid

##### (a) 3-Hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester

3-Hydroxymethyl-piperidine (20.0 g, 0.17 mmol) in acetonitrile was treated with di-*tert*-butyl dicarbonate (37.9 g, 0.17 mol) and DMAP (2.13 g, 1.74 mmol). The reaction mixture was stirred at ambient temperature for 5 h and then concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane/EtOAc, 70:30) to give 3-hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (16.0 g, 44 %).

##### (b) 3-Formyl-piperidine-1-carboxylic acid *tert*-butyl ester

Periodinane (18.2 g, 42.9 mmol) was added to 3-hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (7.10 g, 33.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (230 mL) and stirred for 90 min.

Diethyl ether (230 mL) was added and precipitates were removed by extraction with 10 %

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/saturated NaHCO<sub>3</sub> (1:1, 230 mL). The organic layer was washed with 0.5 M NaOH solution and brine. The organic phase was dried and concentrated under reduced pressure to yield 3-Formyl-piperidine-1-carboxylic acid *tert*-butyl ester (6.50 g, 93 %).

(c) N-Hydroxy-2-piperidin-3-ylmethyl-malonamic acid

The title compound was prepared from 3-formyl-piperidine-1-carboxylic acid *tert*-butyl ester by the method described in Example 5. Yield: (50 %).

5 <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 1.18-1.30 (m, 1H), 1.61-1.99 (m, 6H), 2.64 (t, 1H), 2.86 (t, 1H), 3.20-3.38 (m, 3H).

M (+) 217 (M+1).

Example 7

10 2-(6-Amino-pyridin-3-ylmethyl)-N-hydroxy-malonamic acid.

The title compound was prepared from (5-Formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester by the method described in Example 5. Yield: (23 %).

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 2.99-3.10 (m, 2H), 3.36-4.01 (m, 1H), 6.94 (d, 1H), 7.64 (s, 1H), 7.82 (d, 1H).

15 M (+) 226 (M+1).

Example 8

2-(2-Amino-pyridin-4-ylmethyl)-N-hydroxy-malonamic acid.

20 (a) (4-Formyl-pyridin-2-yl)-carbamic acid tert-butyl ester

(4-Hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (1.91 g, 8.51 mmol) was dissolved in dry DMSO (10 mL) and the reaction flask immersed in a waterbath at 15°C. Triethylamine (1.72 g, 17.0 mmol) was added followed by sulfur trioxide pyridine complex (2.41 g, 15.1 mmol). The reaction mixture was stirred for 2 h and poured onto  
25 crushed ice and the product extracted with CHCl<sub>3</sub>. The organic extract was washed with water, dried concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane/EtOAc, 80:20) to give (4-Formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (1.57 g, 83 %).

30 (b) 2-(2-Amino-pyridin-4-ylmethyl)-N-hydroxy-malonamic acid

The title compound was prepared from (4-Formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester by the method described in Example 5. Yield: (48 %).

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<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 3.10 (dd, 1H), 3.19 (dd, 1H), 3.47 (dd, 1H), 6.77 (d, 1H), 7.82 (s, 1H), 7.71 (d, 1H).  
M (+) 226 (M+1).

5    Example 9

2-[2-(1-*tert*-Butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid.

To a solution of 4-[2-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (17.8 mg, 0.05 mmol) in acetic acid (1 mL) was added 6 M HCl (2 mL). The solution was stirred at room temperature over night and then concentrated  
10 to yield the title compound as the hydrochloric acid salt (15 mg, 100 %).

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 1.30-1.40 (m, 4H), 1.57-1.64 (m, 1H), 1.83-1.90 (m, 1H), 1.90-1.98 (m, 1H), 3.31-3.39 (m, 2H).  
M (+) 216 (M+1).

15    Example 10

2-[2-(1-*tert*-Butoxycarbonyl-piperidin-3-yl)-methyl]-malonic acid.

The title compound was prepared from 3-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-piperidine-1-carboxylic acid *tert*-butyl ester by the method described in Example 9. Yield: (100 %).

20    <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 1.20-1.30 (m, 1H), 1.63-1.76 (m, 1H), 1.78-1.97 (m, 5H), 2.65 (t, 1H), 2.83-2.92 (m, 1H), 3.29-3.38 (m, 2H), 3.42-3.48 (m, 1H).  
M (+) 202 (M+1).

Example 11

25    2-[2-(1-*tert*-Butoxycarbonyl-piperidin-4-yl)-methyl]-malonic acid

(a) Piperidine-1,4-dicarboxylic acid mono-*tert*-butyl ester

Piperidine-4-carboxylic acid (24.5 g, 0.19 mmol) in THF/water (1:1, 417 mL) was treated with di-*tert*-butyl dicarbonate (41.49 g, 0.19 mol) and sodium bicarbonate (16.0 g, 0.19  
30 mol). The reaction mixture was stirred at ambient temperature for 16 h. The THF was then removed under reduced pressure and the aqueous phase washed with dichloromethane. The aqueous layer was then acidified to pH 1-2 with 1 M HCl solution and extracted with ethyl



acetate. The organic phase was washed with brine and dried to give piperidine-1,4-dicarboxylic acid mono-*tert*-butyl ester (35.9 g, 83 %).

(b) 4-Hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester

To a solution of piperidine-1,4-dicarboxylic acid mono-*tert*-butyl ester (19.3 g, 84.0 mmol) in THF (100 mL) was added diborane (185 mL, 1.0 M solution in THF) over a period of 10 min at 0°C. Hydrogen gas was rapidly evolved and after gas evolution had ceased the reaction was stirred at room temperature for 1 h. The reaction mixture was again cooled to 0°C, and 1 M aqueous HCl was added dropwise to the reaction mixture with further evolution of hydrogen. Addition of HCl was continued until the evolution of hydrogen had almost ceased. The mixture was then stirred for 10 min and made basic (pH 13-14) by the addition of 1 M NaOH solution. The aqueous solution was extracted with ethyl acetate, the organic phase washed with brine, dried and concentrated under reduced pressure to yield 4-Hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (18.12 g, 100 %).

(c) 4-Formyl-piperidine-1-carboxylic acid *tert*-butyl ester

Periodinane (26.9 g, 63.5 mmol) was added to 4-Hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (10.5 g, 48.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and stirred for 90 min. Diethyl ether (560 mL) was added and precipitates were removed by extraction with 10 % Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/saturated NaHCO<sub>3</sub> (1:1, 300 mL). The organic layer was washed with 0.5 M NaOH solution and brine. The organic phase was dried and concentrated under reduced pressure. Purification using flash chromatography (hexane/EtOAc, 8:2) gave 4-Formyl-piperidine-1-carboxylic acid *tert*-butyl ester (8.5 g, 81 %).

(d) 2-[2-(1-*tert*-Butoxycarbonyl-piperidin-4-yl)-methyl]-malonic acid.

The title compound was prepared from 4-Formyl-piperidine-1-carboxylic acid *tert*-butyl ester by the method described in Example 5 and 9. Yield: (100 %).

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 1.38-1.48 (m, 2H), 1.61-1.75 (m, 1H), 1.82-1.90 (m, 2H), 1.92-2.02 (m, 2H), 2.90-3.01 (m, 2H), 3.35-3.42 (m, 2H), 3.42-3.48 (m, 1H).

M (+) 202 (M+1).

Example 12

2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid.

The title compound was prepared from [5-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin-2-yl]-carbamic acid *tert*-butyl ester by the method described in Example 9. Yield: (100 %).

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 3.08 (d, 2H), 3.66 (t, 1H), 6.98 (d, 1H), 7.73 (s, 1H), 7.92 (d, 1H).

M (+) 211 (M+1).

Example 132-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid.

The title compound was prepared from [4-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin-2-yl]-carbamic acid *tert*-butyl ester by the method described in Example 9. Yield: (100 %).

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ 3.10 (d, 2H), 3.79 (t, 1H), 6.84 (d, 1H), 7.92 (s, 1H), 7.77 (d, 1H).

M (+) 211 (M+1).

Example 142-(2-Amino-pyridin-4-ylmethyl)-succinic acid(a) 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethylene)-succinic acid 4-benzyl ester 1-*tert*-butyl ester

Butyllithium (1.6 M in hexane, 14.8 ml, 23.7 mmol) was added dropwise to a solution of 2-(diethoxy-phosphoryl)-succinic acid 4-benzyl ester 1-*tert*-butyl ester (9.50 g, 23.7 mmol) in THF (75 mL) at 0°C under nitrogen. After stirring at 0°C for 1 h, the solution was transferred to a solution of (4-Formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (3.70 g, 16.6 mmol) in THF (75 mL). The resulting reaction mixture was stirred at 0°C for 1 h before being allowed to warm to 25 °C, and the mixture was stirred overnight. Water (400 mL) was added and the product extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x50 mL). The combined organic layers were dried and concentrated. Flash chromatography (hexane/EtOAc, 4:1) gave 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethylene)-succinic acid 4-benzyl ester 1-*tert*-butyl ester 4.30 g (55 %).

(b) 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-succinic acid 1-*tert*-butyl ester  
2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethylene)-succinic acid 4-benzyl ester 1-*tert*-  
butyl ester (2.81 g, 7.60 mmol) and Pd/C (10 %, 400 mg) were suspended in EtOH and  
5 hydrogenated at 41 atm. and 28°C for 3 days. The catalyst was removed from the reaction  
mixture by filtration. The catalyst was washed with EtOH (96 %). 1 M K<sub>2</sub>CO<sub>3</sub> (30 mL) was  
added to the filtrate followed by addition of water (50 mL). After 2 days the reaction  
mixture was evaporated to ca 80 mL, then brine (10 mL) was added and the reaction  
mixture extracted with ether. The aqueous phase was acidified to pH=3 and extracted with  
10 chloroform. Methanol (25 mL) was added and the reaction mixture was dried (Na<sub>2</sub>SO<sub>4</sub> +  
CaSO<sub>4</sub>) and filtered to give 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-succinic  
acid 1-*tert*-butyl ester (1.90 g, 83 %).

(c) 2-(2-Amino-pyridin-4-ylmethyl)-succinic acid

15 To a solution of 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-succinic acid 1-*tert*-  
butyl ester (164 mg, 0.43 mmol) in methylene chloride (1.5 mL) was added TFA (1.5 mL).  
The reaction mixture was stirred for 2.5 h and then concentrated under reduced pressure.  
The residue was lyophilized to give the title compound as the TFA salt (126mg, 87 %)  
<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 2.58-2.80 (m, 2H), 2.88-3.07 (m, 2H), 3.13-3.26 (m, 1H),  
20 6.79-6.84 (dd, 1H), 6.84-6.88 (s, 1H), 7.69-7.75 (d, 1H).  
MS (+) 225 (M+1).

Example 15

trans-2-(4-Amino-cyclohexylmethyl)-succinic acid

25 (a) 4-[N-(*tert*-Butoxycarbonyl)amino]-cyclohexane carboxylic acid

To a solution of *cis*-4-aminocyclohexane carboxylic acid (9.90 g, 69.0 mmol) in water (120  
mL) and dioxane (120 mL) was added KOH (3.73 g, 56 mmol) followed by di-*tert*-butyl  
dicarbonate (15.3 g, 70.0 mmol). The reaction mixture was stirred at room temperature  
30 overnight. Water was added and the product was extracted with CHCl<sub>3</sub>. The combined  
organic extracts were washed with water, dried and concentrated under reduced pressure to  
give 4-[N-(*tert*-butoxycarbonyl)amino]-cyclohexane carboxylic acid (14.1 g, 84 %).

(b) [4-(Methoxy-methyl-carbamoyl)-cyclohexyl]-carbamic acid *tert*-butyl ester

A solution of 4-[*N*-(*tert*-butoxycarbonyl)amino]-cyclohexane carboxylic acid (11.95 g, 49.0 mmol), *O,N*-dimethylhydroxylamine (4.88 g, 50.0 mmol), DCC (9.60 g, 50 mmol) and triethylamine (5.06 g, 50.0 mmol) in DMF (150 mL) was stirred at room temperature overnight. Water (500 mL) was added and the mixture was extracted with CHCl<sub>3</sub>. The organic phase was washed with water, dried and concentrated under reduced pressure. Purification by flash chromatography (hexane/EtOAc 1:1) gave [4-(methoxy-methyl-carbamoyl)-cyclohexyl]-carbamic acid *tert*-butyl ester (8.50 g, 61 %).

(c) (4-Formyl-cyclohexyl)-carbamic acid *tert*-butyl ester

[4-(Methoxy-methyl-carbamoyl)-cyclohexyl]-carbamic acid *tert*-butyl ester (7.50 g, 26.2 mmol) in dry ether (150 ml) was reduced with an excess LiAlH<sub>4</sub>. The reaction mixture was quenched by careful addition of water and extracted with CHCl<sub>3</sub>. The mixture was dried and concentrated under reduced pressure to give (4-formyl-cyclohexyl)-carbamic acid *tert*-butyl ester (6.30 g, 93 %).

(d) trans-[4-(Benzylimino-methyl)-cyclohexyl]-carbamic acid *tert*-butyl ester

A mixture of (4-Formyl-cyclohexyl)-carbamic acid *tert*-butyl ester (3.80 g, 16.7 mmol), benzylamine (1.82 g, 16.7 mmol), acetic acid (0.01 g, 16.7 mmol) and anhydrous magnesium sulfate (4.01 g, 33.3 mmol) in methylene chloride (20 mL) was stirred for 5 days. The mixture was filtered through Celite and concentrated under reduced pressure to give trans-[4-(benzylimino-methyl)-cyclohexyl]-carbamic acid *tert*-butyl ester (5.10 g, 97 %) as a 97:3 *trans:cis* mixture.

(e) trans-(4-Formyl-cyclohexyl)-carbamic acid *tert*-butyl ester

A solution of trans-[4-(benzylimino-methyl)-cyclohexyl]-carbamic acid *tert*-butyl ester (2.50 g, 8.00 mmol) and oxalic acid (0.80 g) in water/THF (50 mL, 1:1) was stirred for 10 h at room temperature. The reaction mixture was concentrated under reduced pressure and methylene chloride (50 mL) was added to the residue. The organic phase was dried and concentrated under reduced pressure to give trans-(4-Formyl-cyclohexyl)-carbamic acid *tert*-butyl ester (1.3 g, 80 %).

(f) trans-2-(4-*tert*-Butoxycarbonylamino-cyclohexylmethylene)-succinic acid 4-benzyl ester 1-*tert*-butyl ester

Butyllithium (1.6 M in hexane, 5.0 ml, 8.00 mmol) was added dropwise to a solution of 2-(diethoxy-phosphoryl)-succinic acid 4-benzyl ester 1-*tert*-butyl ester (3.21 g, 8.00 mmol) in THF (25 mL) at 0°C under nitrogen. After stirring at 0°C for 1 h, the solution was transferred to a solution of trans-(4-Formyl-cyclohexyl)-carbamic acid *tert*-butyl ester (1.30 g, 5.72 mmol) in THF (10 mL). The resulting mixture was stirred at 0°C for 1 h and at room temperature overnight. Water was added and the product extracted with CH<sub>2</sub>Cl<sub>2</sub>.

The organic phase was dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 80:20) gave trans-2-(4-*tert*-Butoxycarbonylamino-cyclohexylmethylene)-succinic acid 4-benzyl ester 1-*tert*-butyl ester (1.10 g)

(g) trans-2-(4-*tert*-Butoxycarbonylamino-cyclohexylmethyl)-succinic acid 1-*tert*-butyl ester

A solution of trans-2-(4-*tert*-Butoxycarbonylamino-cyclohexylmethylene)-succinic acid 4-benzyl ester 1-*tert*-butyl ester (243 mg, 0.51 mmol) and palladium (5 % on charcoal) in ethanol (15 mL) was hydrogenated at 4 bar for 3 h. The catalyst was removed from the reaction mixture by filtration. The catalyst was washed with ethanol and the solution was concentrated under reduced pressure to give crude trans-2-(4-*tert*-Butoxycarbonylamino-cyclohexylmethyl)-succinic acid 1-*tert*-butyl ester (217 mg, >100 %).

(h) trans-2-(4-Amino-cyclohexylmethyl)-succinic acid

To a solution of trans-2-(4-*tert*-Butoxycarbonylamino-cyclohexylmethyl)-succinic acid 1-*tert*-butyl ester (200 mg, 0.52 mmol) in methylene chloride (1.32 g, 15.6 mmol) was added triethylsilane (150 mg, 1.30 mmol) followed by TFA (770 mg, 6.75 mmol). The reaction mixture was stirred for 2.5 h and then concentrated under reduced pressure. Purification by HPLC (0-80 % acetonitrile, 0.1 % TFA in water) gave the title compound as the TFA salt (60 mg, 34 %)

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 0.99-1.11 (m, 2H), 1.30-1.46 (m, 4H), 1.54-1.62 (m, 1H), 1.79-1.86 (m, 1H), 1.89-1.96 (m, 1H), 1.99-2.06 (m, 2H), 2.58-2.71 (m, 2H), 2.85-2.95 (m, 1H), 3.08-3.17 (m, 1H).

MS (+) 230 (M+1).

#### Example 16

##### 2-(6-Amino-pyridin-3-ylmethyl)-N-benzyl-N-hydroxy-succinamic acid

5

##### (a) N-Benzyl-N-benzyloxy-2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-succinamic acid tert-butyl ester

To a solution of 2-(2-tert-butoxycarbonylamino-pyridin-4-ylmethyl)-succinic acid 1-tert-butyl ester (0.67 g, 1.76 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was added N-benzyl-N-benzyloxy amine (0.42 g, 1.94 mmol), DCC (0.40 g, 1.94 mmol) and DMAP (0.02 g, 0.17 mmol) and the mixture was stirred overnight. Water was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was dried and filtered and the residue purified by flash chromatography (hexane/EtOAc, 4:1) to give N-Benzyl-N-benzyloxy-2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-succinamic acid tert-butyl ester (0.51 g, 50 %).

15

##### (b) 2-(6-Amino-pyridin-3-ylmethyl)-N-benzyl-N-benzyloxy-succinamic acid

To a solution of N-Benzyl-N-benzyloxy-2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-succinamic acid tert-butyl ester (1.0 g, 1.7 mmol) in methylene chloride (10 mL) was added TFA (4 mL) at 0°C. The reaction mixture was stirred for 4 hs and then concentrated under reduced pressure to give crude 2-(6-Amino-pyridin-3-ylmethyl)-N-benzyl-N-benzyloxy-succinamic acid as the TFA salt (0.9 g, 100 %).

20

##### (c) 2-(6-Amino-pyridin-3-ylmethyl)-N-benzyl-N-hydroxy-succinamic acid

A solution of 2-(6-Amino-pyridin-3-ylmethyl)-N-benzyl-N-benzyloxy-succinamic acid (0.9 g, 1.7 mmol) and palladium (0.5g, 5 % on charcoal) in methanol (100 mL) was hydrogenated at 1 bar for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 10:5:1) to give the title compound (123 mg, 22 %).

25

<sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ 2.50-3.03 (m, 5H), 4.72 (q, 2H), 6.65 (d, 1H), 7.18-7.31 (m, 6H), 7.53 (d, 1H), 7.65 (s, 1H).

30

MS (+) 330 (M+1).

Example 172-(6-amino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid5 (a) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid ethyl ester

A solution of (3-Phenyl-propyl)-phosphinic acid (0.579g 3.143mmol) and 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.327g 1.067 mmol) in MeCN (7.5 mL) was degassed using the freeze-thaw technique. BSA (2.55g 12.57 mmol) was added and the mixture was stirred for 3 days and concentrated under reduced pressure. The residue was dissolved in chloroform, washed with NaHCO<sub>3</sub> and brine, dried and concentrated under reduced pressure. Flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 8:2) gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid ethyl ester (0.240g, 16%).

15 (b) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid

To a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid ethyl ester (0.240g 0.489 mmol) in MeCN (3 mL) was added a solution of LiOH (0.059 g 2.45 mmol) in H<sub>2</sub>O (3 mL). The mixture was then stirred at 20°C for 1.5 hours. Ethylacetate was added and the mixture was washed with 1 M HCL and brine and filtered to give 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid (0.174 g, 77%) as white crystals.

25 (c) 2-(6-Amino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid

To a mixture of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-3-[hydroxy-(3-phenyl-propyl)-phosphinoyl]-propionic acid ( 0.170 g, 0.367 mmol) in ethylacetate (3 mL) at 4°C was slowly added ethylacetat (4 mL, saturated with HCl(g)). The mixture was then stirred for 22 h. Concentration under reduced pressure gave the title compound (0.124 g, 93%) as the hydrochloride salt.

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$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{SOCD}_3$ ):  $\delta$  1.52-1.72 (m, 4H), 1.88-1.96 (m, 2H), 2.47-2.49 (t, 1H), 2.57-2.62 (m, 2H), 2.77-2.82 (m, 2H), 6.94-6.97 (d, 1H), 7.15-7.29 (m, 4H), 7.75-7.80 (m, 2H), 8.05 (s, 1H)

MS (+) 363 (M+1).

5

### Example 18

2-(6-Amino-5-methyl-pyridin-3-ylmethyl)-3-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyl]-propionic acid

- 10 (a) 2-[(1-Benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoylmethyl]-3-(6-bis(tert-butoxycarbonyl)amino-5-methyl-pyridin-3-yl)-propionic acid ethyl ester

A solution of 2-(6-bis(tert-butoxycarbonyl)amino-5-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.6 g, 1.43 mmol) and (1-benzyloxycarbonylamino-2-methyl-propyl)-phosphinic acid (0.678 g, 2.5 mmol) in dry acetonitrile (10 mL) was degassed using the freeze-thaw technique. Bistrimethylsilylacetamide (5 mL, 20.3 mmol) was added under argon. The resulting mixture was stirred at room temperature for 84 h and then concentrated under reduced pressure. The remaining mixture was dissolved in chloroform and washed with saturated aqueous  $\text{NaHCO}_3$ . The aqueous phase was extracted with chloroform and EtOAc. The combined organic extracts were dried and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/EtOH, 100:20  $\rightarrow$  100:25) to yield 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoylmethyl]-3-(6-bis(tert-butoxycarbonyl)amino-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (650 mg, 65,9%)

- 25 (b) 2-[(1-Benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoylmethyl]-3-(6-tert-butoxycarbonylamino-5-methyl-pyridin-3-yl)-propionic acid

1 M LiOH (2 mL) was added dropwise to a solution of 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoylmethyl]-3-(6-bis(tert-butoxycarbonyl)amino-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (100 mg, 0.145 mmol) in acetonitrile (2 mL). The mixture was stirred overnight and concentrated under reduced pressure. The mixture was purified by column chromatography (isopropanol/concentrated aqueous  $\text{NH}_3$ /water, 4:2:1) to give unpure product. The unpure product was stirred with MeOH,

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filtered and concentrated under reduced pressure. The crude product was stirred with EtOH, filtered and concentrated under reduced pressure. The crude product was stirred with ethylacetate/ethanol, filtered and concentrated under reduced pressure to give 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoylmethyl]-3-(6-tert-butoxycarbonylamino-5-methyl-pyridin-3-yl)-propionic acid (52 mg, 63.8 %).

(c) 2-(6-Amino-5-methyl-pyridin-3-ylmethyl)-3-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoyl]-propionic acid

A solution of 2-[(1-benzyloxycarbonylamino-2-methyl-propyl)-hydroxy-phosphinoylmethyl]-3-(6-tert-butoxycarbonylamino-5-methyl-pyridin-3-yl)-propionic acid (52 mg, 0.09 mmol) and ethylacetate (6 mL, saturated with HCl (g)) was stirred for 20 min. The mixture was concentrated under reduced pressure, washed with acetonitrile and dissolved in ethanol and petroleumether. The solution was concentrated under reduced pressure to give the title compound (41 mg, 91.3 %) as the hydrochloride salt.

<sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O): δ 0.99-1.05 (m, 6H), 1.75-1.85 (m, 1H), 2.10-2.28 (m, 5H) 2.68-3.15 (m, 3H), 3.75-3.81 (m, 1H), 5.04-5.19 (m, 2H), 7.20-7.37 (m, 5H), 7.51-7.71 (m, 2H).

MS (+) 501 (M+1)

Example 19

2-(6-Amino-pyridin-3-ylmethyl)-3-methyl-succinic acid

(a) 2-Ethoxycarbonyl-3-methyl-succinic acid diethyl ester

A solution of diethylmalonate (2.44g, 15 mmol) and cesium fluoride (2.32g, 15mmol) in DMF (20 mL) was stirred for 1 h at room temperature under argon. A solution of 2-methanesulfonyloxy-propionic acid ethyl ester (1.0 g, 5 mmol) in DMF (5 mL) was added and the mixture was stirred at 45°C overnight. The mixture was poured into H<sub>2</sub>O and extracted with ethyl acetate. The organic layers were washed with water, dried, and concentrated under reduced pressure. The crude product was purified by vacuum distillation. The product was purified by column chromatography (petroleumether/ethyl acetate, 4:1) to give 2-(6-amino-pyridin-3-ylmethyl)-3-methyl-succinic acid (0.5g, 37.7%).

(b) 2-(6-tert-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethoxycarbonyl-3-methyl-succinic acid diethyl ester

A solution of 2-(6-amino-pyridin-3-ylmethyl)-3-methyl-succinic acid (300mg, 1.15 mmol) and NaH (30 mg, 1.25 mmol) in DMF (1 mL) was stirred at room temperature for 1 h under argon. A solution of (5-bromomethyl-pyridin-2-yl)-carbamic acid tert-butyl ester (330 mg, 1.15 mmol) in DMF (1 mL) was added and the reaction mixture was stirred for 24 h. The reaction was poured into H<sub>2</sub>O and extracted with ethyl acetate. The organic layer was washed with water, dried and concentrated under reduced pressure. The product was purified by column chromatography (petroleumether/ethyl acetate, 4:1) to give 2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethoxycarbonyl-3-methyl-succinic acid diethyl ester (220 mg, 40.9%).

(c) 2-(6-Amino-pyridin-3-ylmethyl)-3-methyl-succinic acid

A solution of 2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethoxycarbonyl-3-methyl-succinic acid diethyl ester (94 mg, 0.20 mmol) in concentrated aqueous HCl (4 mL) was stirred at reflux overnight. The mixture was concentrated under reduced pressure and freeze dried to give the title compound as the HCl salt (50mg, 90.3%)

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 1.22-1.35 (m, 3H), 2.77-3.08 (m, 4H), 6.99-7.05 (d, 1H), 7.70 (s, 1H), 7.84-7.91 (m, 1H).

MS (+) 239 (M+1)

Example 20

2-(6-Amino-pyridin-3-ylmethyl)-3-phenethyl-succinic acid

(a) 2-Ethoxycarbonyl-3-phenethyl-succinic acid diethyl ester

A solution of diethylmalonate (240.2 mg, 1.5 mmol) and NaH (43.2 mg, 1.8 mmol) in THF (3 ml) was stirred for one hour at room temperature under argon. 2-(4-Nitro-benzenesulfonyloxy)-4-phenyl-butyric acid ethyl ester (590 mg, 1.5 mmol) and DMPU (192.2 mg, 1.5 mmol) was added and the mixture was stirred at room temperature for three days. The reaction was poured into H<sub>2</sub>O and extracted with ethyl acetate. The organic layer was washed with water and concentrated under reduced pressure. The product was purified

by column chromatography (petroleum ether/ethyl acetate, 4:1) to give 2-ethoxycarbonyl-3-phenethyl-succinic acid diethyl ester (274 mg, 52.1%).

(b) 2-(6-tert-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethoxycarbonyl-3-phenethyl-succinic acid diethyl ester

A solution of 2-(6-amino-pyridin-3-ylmethyl)-3-phenethyl-succinic acid (9.3 mg, 0.027 mmol) and NaH (1.39 mg, 0.032 mmol) in DMF (0.5 ml) was stirred for 15 minutes at room temperature. (5-Bromomethyl-pyridin-2-yl)-carbamic acid tert-butyl ester (8.76 mg, 0.031 mmol) was added and the mixture was stirred at room temperature over night. The reaction was poured into H<sub>2</sub>O and extracted with ethyl acetate. The organic layer was washed with water and concentrated under reduced pressure. The product was purified by column chromatography (heptane/ethyl acetate, 2:1) to give 2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethoxycarbonyl-3-phenethyl-succinic acid diethyl ester (3.8 mg, 25.7 %).

(c) 2-(6-Amino-pyridin-3-ylmethyl)-3-phenethyl-succinic acid

A solution of 2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethoxycarbonyl-3-phenethyl-succinic acid diethyl ester (94 mg, 0.169 mmol) in concentrated HCl (7 ml) was stirred at reflux for 24 hours. The mixture was concentrated under reduced pressure and freeze dried to give the title compound as the HCl salt (55 mg, 99.2 %).

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 1.63-1.98 (m, 2H), 2.42-2.85 (m, 4H), 3.00-3.60(m, 2H), 6.85-6.98 (m, 1H), 7.10-7.29 (m, 5H), 7.76-8.05 (m, 2H).

MS (+) 329 (M+1)

Example 21

2-(6-Amino-pyridin-3-ylmethyl)-3-butyl-succinic acid

(a) 2-Butyl-3-ethoxycarbonyl-succinic acid diethyl ester

A solution of diethylmalonate (3.589 g, 0.022 mol) and cesium fluoride (3.406 g, 0.024 mol) in DMF (50 ml) was stirred for one hour at room temperature under argon. 2-Bromohexanoic acid ethyl ester (5 g, 0.022 mol) was added and the mixture was stirred at 100 °C and then at 65°C over night. The reaction was poured into H<sub>2</sub>O and extracted with ethyl

acetate. The organic layer was washed with water and concentrated under reduced pressure. The product was purified by column chromatography (heptane/ethyl acetate, 1:2) to give 2-butyl-3-ethoxycarbonyl-succinic acid diethyl ester (5.0 g, 73.8 %).

5 (b) 2-(6-tert-Butoxycarbonylamino-pyridin-3-ylmethyl)-3-butyl-2-ethoxycarbonyl-succinic acid diethyl ester

A solution of 2-butyl-3-ethoxycarbonyl-succinic acid diethyl ester (1 g, 3 mmol) and NaH (119 mg, 5 mmol) in DMF (20 ml) was stirred for 60 minutes at 0°C under argon. (5-Bromomethyl-pyridin-2-yl)-carbamic acid tert-butyl ester (1.425 g, 5 mmol) was added  
10 and the mixture was stirred at room temperature for one week. Ethanol (1 ml) was added and the reaction was poured into H<sub>2</sub>O and extracted with ethyl acetate. The organic layer was washed with water and concentrated under reduced pressure. The product was purified by column chromatography (heptane/ethyl acetate, 4:1 to 1:1) to give 2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-3-butyl-2-ethoxycarbonyl-succinic acid diethyl  
15 ester (1.2 g, 71.3 %).

(c) 2-(6-Amino-pyridin-3-ylmethyl)-3-butyl-succinic acid

A solution of 2-(6-tert-butoxycarbonylamino-pyridin-3-ylmethyl)-3-butyl-2-ethoxycarbonyl-succinic acid diethyl ester (50 mg, 0.098 mmol) in concentrated HCl (4  
20 ml) was stirred at reflux for 24 hours. The mixture was concentrated under reduced pressure and freeze dried to give the title compound as the HCl salt (28 mg, 89.9 %).

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 0.83-0.91 (m, 3H), 1.18-1.40 (m, 4H), 1.50-1.82 (m, 2H), 2.67-2.75 (m, 12H), 2.78-2.85 (m, 2H), 2.89-3.03 (m, 1H), 6.97-7.09 (m, 1H), 7.63-7.67 (m, 1H), 7.80-7.85 (m, 1H).

25 MS (+) 281 (M+1)

Abbreviations

Ac = acetate

aq = aqueous

30 AIBN = α,α'-azoisobutyronitrile

Bn = benzyl

BSA = N,O-bis(trimethylsilyl)acetamide

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Bu = butyl

Bz = benzoyl

DCC = dicyclohexylcarbodiimide

DIAD = diisopropyl azodicarboxylate

5 DIPEA = diisopropylethylamine

DMAP = N,N-dimethyl amino pyridine

DME = 1,2-dimethoxyethane

DMF = dimethylformamide

DMSO = dimethylsulfoxide

10 EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

Et = ethyl

EtOAc = ethyl acetate

EtOH = ethanol

h = hour

15 HMDS = hexamethyldisilazane

HOAc = acetic acid

HOBt = 1-hydroxybenzotriazol

HPLC = high performance liquid chromatography

KHMDS = potassium bis(trimethylsilyl)amide

20 LDA = lithium diisopropylamide

Me = methyl

MeOH = methanol

min = minutes

PMB = 4-methoxybenzyl

25 Ph = phenyl

Pr = propyl

PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

TEA = triethylamine

TFA = trifluoroacetic acid

30 THF = tetrahydrofuran

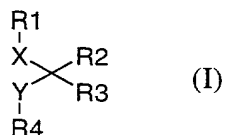
TMSCN = trimethylsilyl cyanide

Tos = toluene-4-sulfonyl

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## CLAIMS

1. A compound of general Formula I



or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

$R_1$  represents,

$C_1$ - $C_6$  alkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O,

and substituted with one or more basic groups such as amino, amidino and/or guanidino;

or aryl, substituted with one or more basic groups such as amino, amidino and/or guanidino,

$R_2$  represents H, acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl,

aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, cyano, cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol,  $Z_2N$ -CO-O-, ZO-CO-NZ- or  $Z_2N$ -CO-NZ- group,

$R_3$  represents  $COOR_5$ ,  $SO(OR_5)$ ,  $SO_3R_5$ ,  $P=O(OR_5)_2$ ,  $B(OR_5)_2$ ,  $P=OR_5(OR_5)$ , or tetrazole, or any carboxylic acid isostere,

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R<sub>4</sub> represents a  $\begin{array}{c} \text{O}-\text{R}_5 \\ | \\ -\text{P}-\text{R}_6 \\ || \\ \text{O} \end{array}$  -group, or a  $\begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{N}-\text{OH} \\ | \\ \text{R}_7 \end{array}$  -group, or a  $\begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{O}-\text{R}_5 \end{array}$  -group,

R<sub>5</sub> represents H, C<sub>1</sub>-C<sub>6</sub> alkyl or aryl,

R<sub>6</sub> represents C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl, heterocyclyl, or an optionally N-substituted  
H<sub>2</sub>N-C(Z)-CONH-C(Z)- or H<sub>2</sub>N-C(Z)- group,

5 R<sub>7</sub> represents H or C<sub>1</sub>-C<sub>6</sub> alkyl,

X represents O, S, SO, SO<sub>2</sub>, C(Z)<sub>2</sub>, N(Z), NR<sub>7</sub>SO<sub>2</sub>, SO<sub>2</sub>NR<sub>7</sub>, NR<sub>7</sub>CO or CONR<sub>7</sub>,

Y represents O, N(Z), S, C(Z)<sub>2</sub>, or a single bond,

Z represents independently H, C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl or heterocyclyl,

with the proviso that when X represents O, S, SO, SO<sub>2</sub>, N(Z), NR<sub>7</sub>SO<sub>2</sub>, SO<sub>2</sub>NR<sub>7</sub>, or

10 NR<sub>7</sub>CO then Y represents C(Z)<sub>2</sub> or a single bond.

2. The compound according to claim 1, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt,

wherein

15 R<sub>1</sub> represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O, and substituted

20 with one or more basic groups such as amino, amidino and/or guanidino;

R<sub>2</sub> represents H, C<sub>1</sub>-C<sub>3</sub> alkyl, amino, halogen, hydroxy,

R<sub>3</sub> represents COOR<sub>5</sub>,

R<sub>4</sub> represents a  $\begin{array}{c} \text{O}-\text{R}_5 \\ | \\ -\text{P}-\text{R}_6 \\ || \\ \text{O} \end{array}$  -group,

25 R<sub>5</sub> represents H, C<sub>1</sub>-C<sub>6</sub> alkyl or aryl,

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$R_6$  represents  $C_1$ - $C_6$  alkyl, aryl, cycloalkyl, heterocyclyl, or an optionally N-substituted

$H_2N-C(Z)-CONH-C(Z)-$  or  $H_2N-C(Z)-$  group,

X represents  $C(Z)_2$ ,

Y represents O or  $C(Z)_2$ ,

5 Z represents independently H or  $C_1$ - $C_6$  alkyl.

3. The compound according to claim 1, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt,

wherein

10  $R_1$  represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

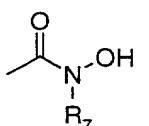
heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O, and substituted

15 with one or more basic groups such as amino, amidino and/or guanidino;

$R_2$  represents H,  $C_1$ - $C_3$  alkyl, amino, halogen or hydroxy,

$R_3$  represents  $COOR_5$ ,

$R_4$  represents a  -group,

$R_5$  represents H,  $C_1$ - $C_6$  alkyl or aryl,

20  $R_7$  represents H or  $C_1$ - $C_6$  alkyl,

X represents  $C(Z)_2$ ,

Y represents  $C(Z)_2$ , or a single bond,

Z represents independently H or  $C_1$ - $C_6$  alkyl.

25 4. The compound according to claim 1, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt,

wherein

$R_1$  represents,



cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;


heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O,

5 and substituted with one or more basic groups such as amino, amidino and/or  
guanidino;

R<sub>2</sub> represents H, C<sub>1</sub>-C<sub>3</sub> alkyl, amino, halogen or hydroxy,

R<sub>3</sub> represents COOR<sub>5</sub>.

R<sub>4</sub> represents a -group,

R<sub>5</sub> represents H, C<sub>1</sub>-C<sub>6</sub> alkyl or aryl,

$X$  represents  $C(Z)_2$ ,

Y represents  $C(Z)_2$ , or a single bond,

Z represents independently H or C<sub>1</sub>-C<sub>6</sub> alkyl.

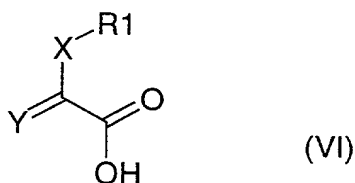
5. A process for the preparation of a compound according to any one of claims 1-4, wherein R<sub>1</sub>, R<sub>5</sub>, R<sub>6</sub>, and Z are as defined in claim 1 and R<sub>2</sub> is H, R<sub>3</sub> is COOR<sub>5</sub>,

$R_4$  represents a  $\begin{array}{c} \text{O}-R_5 \\ | \\ -\text{P}-R_6 \\ || \\ \text{O} \end{array}$  -group,

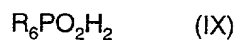
$X$  is  $C(Z)_2$ ,  $Y$  is  $C(Z)_2$ .

comprising the step of:

20 reacting a compound of Formula VI,



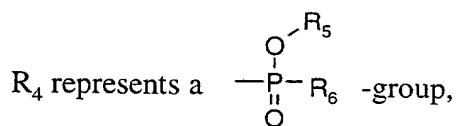
wherein  $\text{R}_1$  and  $\text{Z}$  is as defined in claim 1 and  $\text{X}$  is  $\text{C}(\text{Z})_2$  and  $\text{Y}$  is  $\text{C}(\text{Z})_2$  with a compound of Formula IX,



wherein  $\text{R}_6$  is as defined in claim 1, in the presence of a suitable reagent, such as BSA or HMDS, under standard conditions.

6. A process for the preparation of a compound according to any one of claims 1-4,

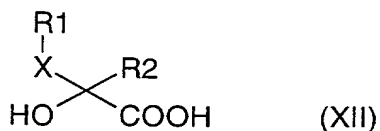
wherein  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_5$ ,  $\text{R}_6$ , and  $\text{Z}$  are as defined in claim 1,  $\text{R}_3$  is  $\text{COOR}_5$ ,  $\text{X}$  is  $\text{C}(\text{Z})_2$ ,  $\text{Y}$  is  $\text{O}$ ,



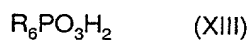
and

comprising the step of:

reacting a compound of Formula XII,



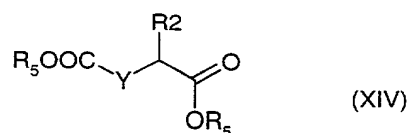
wherein  $\text{R}_1$  and  $\text{R}_2$  are as defined in claim 1 and  $\text{X}$  is  $\text{C}(\text{Z})_2$  with a compound of Formula XIII,



wherein  $R_6$  is as defined in claim 1, in the presence of suitable coupling reagents such as DCC/DMAP, PyBop/DIPEA or  $\text{SOCl}_2$ , under standard conditions.

7. A process for the preparation of a compound according to any one of claims 1-4,

- 5 wherein  $R_1$  and  $R_2$  are as defined in claim 1 and X and Y is  $\text{C}(\text{Z})_2$  or a single bond and  $R_3$  and  $R_4$  are  $\text{COOR}_5$ , comprising the step of:  
reacting a compound of Formula XIV,



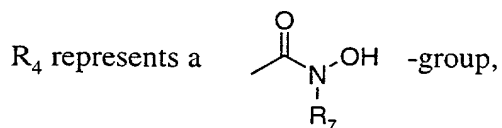
- 10 wherein  $R_2$  and  $R_5$  are as defined in claim 1 and Y is  $\text{C}(\text{Z})_2$  or a single bond, with a compound of the general Formula III,



- 15 wherein  $R_1$  is as defined in claim 1, X is  $\text{C}(\text{Z})_2$  and L is a suitable leaving group, such as Cl, Br, I or tosyl, in the presence of a suitable base, such as LDA or NaH, under standard conditions.

8. A process for the preparation of a compound according to any one of claims 1-4,

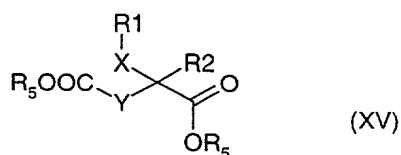
wherein  $R_1$ ,  $R_2$ ,  $R_5$ ,  $R_7$ , X, Y and Z are as defined in claim 1,  $R_3$  is  $\text{COOR}_5$  and



20

comprising the step of:

reacting a compound of Formula XV,



with a compound of Formula XVI,



wherein  $\text{R}_7$  is as defined in claim 1, in the presence of suitable reagents, such as DCC/DMAP, under standard conditions.

9. A pharmaceutical formulation containing a compound according to any one of claims 1-4 as active ingredient in combination with a pharmaceutically acceptable adjuvant, diluent or carrier.

10. The use of a compound according to any one of claims 1 to 4 in therapy.

11. The use of a compound according to any one of claims 1 to 4 for the manufacture of a medicament for the inhibition of carboxypeptidase U.

12. A method for treatment or prophylaxis of conditions associated with inhibition of carboxypeptidase U, comprising administering to a mammal, including man, in need of such treatment an effective amount of a compound as defined in any of claims 1-4.

13. A pharmaceutical formulation for use in the treatment or prophylaxis of conditions associated with inhibition of carboxypeptidase U, comprising a compound as defined in any one of claims 1-4 in combination with a pharmaceutically acceptable adjuvant, diluent or carrier.

14. A pharmaceutical formulation, comprising:

- (i) a compound of Formula I or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, and
- (ii) one or more antithrombotic agent with a different mechanism of action, such as an antiplatelet agent, thromboxane receptor inhibitor, synthetase inhibitor, fibrinogen receptor antagonist, prostacyclin mimetic, phosphodiesterase inhibitor or ADP-receptor ( $P_2T$ ) antagonist,
- in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

15. A kit of parts comprising:

- (i) a pharmaceutical formulation containing a compound of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; and
- (ii) a pharmaceutical formulation containing one or more antithrombotic agent with a different mechanism of action, such as an antiplatelet agent, thromboxane receptor inhibitor, synthetase inhibitor, fibrinogen receptor antagonist, prostacyclin mimetic, phosphodiesterase inhibitor or ADP-receptor ( $P_2T$ ) antagonist, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier;

which compound (i) and agent (ii) are each provided in a form that is suitable for administration in conjunction with the other.

16. A method for treatment of a patient suffering from, or susceptible to, a condition in which inhibition of carboxypeptidase U and a different antithrombotic mechanism are required or desired, which method comprises administering to the patient a therapeutically effective total amount of

- (i) a compound of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with
- (ii) one or more antithrombotic agent with a different mechanism of action, such as an antiplatelet agent, thromboxane receptor inhibitor, synthetase inhibitor, fibrinogen receptor antagonist, prostacyclin mimetic, phosphodiesterase inhibitor or ADP-receptor ( $P_2T$ ) antagonist,

in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

17. A method for treatment of a patient suffering from, or susceptible to, a condition in which inhibition of carboxypeptidase U and a different antithrombotic mechanism are required or desired, which method comprises administering to the patient a formulation as defined in claim 15.

09500660.072000

**DECLARATION FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled NEW COMPOUNDS

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 3 May 2000 as United States Application Number or PCT International Application Number PCT/SE00/00846 and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

9901572-9  
(Number)

Sweden  
(Country)

3 May 1999  
(Day/Month/Year Filed)



\_\_\_\_\_  
(Number)

\_\_\_\_\_  
(Country)

\_\_\_\_\_  
(Day/Month/Year Filed)



I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application Number)

(Filing Date)

(Status -- patented, pending, abandoned)

(Application Number)

(Filing Date)

(Status -- patented, pending, abandoned)

14  
I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Dimitrios Drivas, Reg. No. 32,218; Cecilia O'Brien Lofters, Reg. No. 33,434; Warren S. Heit, Reg. No. 36,828; David Bender, Reg. No. 35,445; John M. Genova, Reg. No. 32,224; Richard J. Sterner, Reg. No. 35,372; Hans-Peter G. Hoffmann, Reg. No. 37,352; Leslie Morioka, Reg. No. 40,304; John Scheibeler, Reg. No. 35,346; Thelma A. Chen Cleland, Reg. No. 40,948; Jean E. Shimotake, Reg. No. 36,273; Jeff Oelke, Reg. No. 37,409; Chase Romick, Reg. No. 45,051; and Louis S. Silvestri, Reg. No. 45,108 of the firm of WHITE & CASE LLP, with offices at 1155 Avenue of the Americas, New York, New York 10036,

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believe to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Marcel Linschoten

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Second inventor's signature

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